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ORIGINAL ARTICLE

Application of Polylactic Acid/Roselle Calyx Extrcat/Halloysite Nanoclay film for Improving Sensory and Chemical Quality of Almond Kernel

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K E Y W O R D S

Antifungal;
Biodegradable film;
Lipid oxidation;
Preservation;
Sensory attributed

A B S T R A C T

The chemical quality of almonds, one of the major nuts of Iran, will be decreased during conservation due to oil oxidation and mold spoilage. The presentation work proposed to study the capability of roselle calyx extract (RCE) and halloysite nano clay (HNC) loaded in polylactic acid (PLA) to control oxidation reactions and fungi growth in almond kernels during storage at 25°C. The PLA biofilms with RCE and HNC/RCE were created using casting method. The total yeast and mold count, thiobarbituric acid (TBA), peroxide value (PV), and sensory properties of almonds were measured during 16 weeks of preservation. On the 16th week, PV was measured as 1.07 meqo$_2$ kg^{-1} oil in almonds whereas the PV of almond kernels was evaluated as 0.78 meqo$_2$ kg^{-1} oil packaged with PLA/RCE/HNC. At 16 weeks, the TBA value was measured as 0.056 mg MDA kg^{-1} packaged PLA films, whereas the TBA value was detected 0.036 MDA kg^{-1} in almonds packaged with RCE/HNC. The total yeast and mold count value of almond kernels packed with neat sample reached 4.12 log CFU g^{-1}, and the lowest contamination was observed in almonds packed with RCE/HNC. At last preservation, by addition of RCE/HNC, the color score was decreased from 5 to 4.5. The mentioned index in the PLA specimens was 3. Our findings represent that PLA/RCE/HNC can be utilized to enhance the quality of almond kernels during storage time.

Introduction

Almond (*Prunus amygdalus*) is a well-known oldest dried fruit and is one of the fruit trees in the majority of temperate regions (Imani *et al*., 2021; Massantini and Frangipane, 2022). Almonds are cultivated in the southern shore of the Mediterranean Sea and West Asia (Ansari and Gharaghani, 2019; Gaglio *et al*., 2023). Almonds are a fine resource of nutritional compounds including proteins, lipids, vitamins (B$_1$, B$_2$, B$_3$, B$_9$, and E) minerals (Fe, Zn, Mn, Cu, P, K, Mg, and Ca), carbohydrates, and phytochemical compounds (phytosterols, squalene, tocopherols, stanols, phospholipids, sphingolipids, chlorophylls, phenols, and carotenoids) (Ansari and Gharaghani, 2019; Vatansever *et al*., 2019). Lipid oxidation is the main cause of decreasing the quality of nut trees

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(Habibi *et al.*, 2023). It creates unpleasant tastes and decreases vitamins and, some pigments (Marvizadeh *et al*., 2014, Sheykholeslami *et al*., 2024). Almond kernels contain 55–70% oil, with good health impacts but are very sensitive to lipid oxidation. Almond kernel oil is a rich resource in monounsaturated (60%) and polyunsaturated (30%) fatty acids (Roncero *et al*., 2020). Micronutrients including Fe, Mn, Cu, antioxidants, water activity, and unsaturated fatty acids influence the oxidative stability of foods (Machado *et al*., 2023). Temperature, humidity, and O_2 availability, are the major external indices that affect almond preservation (Adiletta *et al*., 2020). Hence, oxidative stability can increase due to the application of active film with nano-additive and decreased lipid oxidation (Marvizadeh *et al*., 2017).

Based on the solution mentioned, bionanocomposite film packaging is a bold purpose in packaging. The aim of packaging with nanofillers in food is to prolong preservation by decreasing microbial and mold population, undesirable chemical reactions, and gas transfer (Marvizadeh *et al*., 2021, Nobari *et al*., 2022). Nanoparticles such as nanocellulose, TiO_2, ZnO, Ag, and SiO_2 have antifungal attributes and can also change the barrier characteristics of packaging to prevent O_2 molecule travel (Javidi *et al*., 2022, Vahedi *et al*., 2022).

Kazemi *et al*. (2020) illustrated that the nano-ZnO has antifungal behavior to control the population of A. flavus and its aflatoxin B_1. Mahdavi-Yekta *et al*. (2024) investigated the impact of quinoa peptide and nano-Ag in polyethylene film, on the aflatoxin of pistachio during 30 days of storage. They reported that Aflatoxin B_1 of fresh pistachios was significantly less than in other pistachio kernels when employing the polyethylene containing 35% nanofiller and quinoa peptide.

Although nonbiodegradable polymers applied in the packaging protect foods against contamination and oxidation reactions, the most important challenge is poor recyclability of them (Velásquez *et al*., 2021).

Polylactic acid is used as a biocompatible material for film packaging due to its fine transparency, high tensile strength, and low price (Vatansever *et al*., 2019). Polylactic acid is made from lactic acid, which is produced using of lactic acid of sugarcane or corn (Andrade *et al*., 2023). Brittleness, low thermal stability, and poor barrier behavior decrease the consumption of PLA in film packaging (Roy and Rhim, 2020).

The active agents are used to improve the physicochemical and antibacterial characteristics of biofilms based on PLA (Moosavian *et al*., 2017). Various active compounds including essential oils, and herbal extracts, and nanofillers such as Ag, nano clay, ZnO, TiO_2, nanocellulose have been utilized to improve the antibacterial, chemical, and physical behavior of films based on PLA (Chavoshi *et al*., 2023).

Natural pigments can be utilized in various applications due to their desirable characteristics including; antifungal behavior, antioxidant properties, and absorption of visible light (NANSU *et al*., 2021).

The majority of the constituents in the RCE and sappan heartwood are flavonoid compounds. The herb roselle is widely cultivated in Thailand and other Southeast Asian countries and is well-known internationally. The primary component of RCE is the pH-sensitive pigment categorized as anthocyanin. The anthocyanin pigment in *Hibiscus sabdariffa* changes chemically to create a flavylium cation, which is the reason the plant is red (Etemadi Razlighi *et al*., 2023). Habashi *et al*. (2019) studied the impact of employing various amounts of chitosan/thyme essential oil, on the chemical properties of walnuts during 120 days. They demonstrated that the sample packed with chitosan/thyme essential oil has low levels of free fatty acids (FFAs) compared to the neat specimens. Studies have shown that roselle monosaccharides can enhance athletic performance over a long period. This has to do with how monosaccharides molecules impact skeletal muscle metabolism which can change function (Sadeghi *et al*., 2022).

In this project, active films based on poly lactic acid, poly lactic acid/roselle calyx extract, and poly lactic acid/roselle calyx extract (RCE)/HNC were prepared. The purpose of the current investigation was to assay the changes in thiobarbituric Acid, peroxide value, sensory, and fungal contamination of packaged almonds stored at 25°C.

Materials and Methods

Roselle calyx extract

Extraction of the roselle extract followed the modified method described in Giusti, *et al*.'s [13]. The final mixture was prepared with around 15 mL of ethanol (80%) and 1 g of roselle calyx powder, the pH was adjusted to 2 using HCl. To obtain the last extract, the specimens were heated at 50°C for 50 minutes and then centrifuged at 3000 rpm for 5 minutes. At 50°C, the solvent was removed by a Rotary Evaporator (Heidolph, GER).

Film preparation

Biodegradable films based on polylactic acid (PLA)/roselle calyx extract (RCE)/halloysite nanoclay (HNC) were fabricated using of the technique stated by Rhim *et al*. (2006) with slight modification. About 5 g of PLA resins were added to 100 ml of chloroform under vigorous stirring during 4h. Briefly, chloroform (5 mL) was mixed with RCE (0.5 g) and dispersed in an ultrasonic device during 50 min. The film based on PLA/HNC was obtained by adding 1 (%w/w) of nanoadditive to the PLA solution. PLA/HNC biomixture was agitated at 25°C during 16h. The suspensions were submerged in an ultrasonic device during 55 min. PLA/HNC mixture was added to the RCE solution. The final solution was poured on casting plates and dried at 70°C during 40 min. PLA Film as control sample was fabricated without RCE/HNC.

Hazelnuts specimen packaging

Almond samples obtained from local producer of Sabzevar, Iran, were dehulled and the whole almond were dried using a dryer (UF 110 Memmert Co., Forsthof, GER) at 44 °C till a moisture content of 5% was obtained (Helrich, 1990). Also, the almond samples were crushed, to separate the shell, and peeled, and healthy almond kernels were collected. The kernel samples were then weighed and transferred in lattice containers.

About 25g of almonds were placed between active sheets, using a heat sealer, and conserved at 20°C for 16 weeks. Almonds kernels were measured on the first day and every 4 weeks during conservation.

Peroxide value (PV)

The lipids oxidation was checked using the peroxide value (PV) method. Briefly, 5 g of specimen were mixed with acetic acid-chloroform (30 cm^3) and kept under vigorous stirring at 25°C for 15 min. Then, KI saturated (0.5 cm^3) was mixed with suspension at room temperature. Finally, distilled water was added to solution and titration was carried out with 0.1 N sodium hydroxide.

Thiobarbituric Acid (TBA)

The assay was performed according to Pfalzgraf *et al*. (1995) with slight modification. About, 10 g of sample were added to 10 µg g^{-1} trichloroacetic acid (20 mL), and then homogenized with hot plate (PIT 300, Shimibio .Co, Tehran, Iran). The solution was filtered using tissue paper. After that, 2 mL of the solution were added to 20 mM thiobarbituric acid (2 mL). The solution was placed in a thermostatic bath for 20 min at 97°C. The absorbance of the sample was evaluated at 532 nm for the blank specimen.

Total yeast and mold counts

About, 10 g of sample was pounded with a sterile mortar. Almond kernel powder, along with 0.9% sterilized Hcl (90 mL), was placed in stomacher bag and mixed in the stomacher (Sientz-04 Ningbo Scientz Biotechnology Co.Ltd, China).

Different dilutions such as 10^{-2} and 10^{-3} were prepared from dilutions of 10^{-1}. Approximately 0.1 mL of each dilution was added to the PCA agar spread on the culture medium and incubated at 20°C during 5 days.

Sensory properties

The sensory attributes of almonds were performed by 9 panelists on the first and 16th week of the preservation. They scored color and flavor factors from 1 (extremely dislike) to 5 (extremely like). Also, six almond kernels were placed on dishes and proposed to 9 panelists to measure sensory properties.

Statistical analysis

ANOVA test were used to compare data of sensory properties, TBA, PV, total yeast and mold counts of almonds at $p<0.05$. Data of each of the test was analyzed by Graph Pad Prism 9.5.1.733.

Results

Peroxide value

Change in PV are represented in Fig. 1. The conservation time and PLA film containing RCE and RCE/HNC, significantly ($p < 0.05$) affected PV. Significant differences ($p < 0.05$) were found in the PV of almonds with active biofilms with RCE and RCE/HNC compared with the control packaging during the preservation. After 16 weeks of preservation, PV reached 1.07 meqo_2 kg^{-1} oil for almonds packed with PLA films. Almonds packaged with HNC/RCE had very low PV, 0.78 meqo_2 kg^{-1} oil in almonds. Moreover, the PV of almonds packaged with PLA film was higher than that packaged with RCE and RCE/HNC.

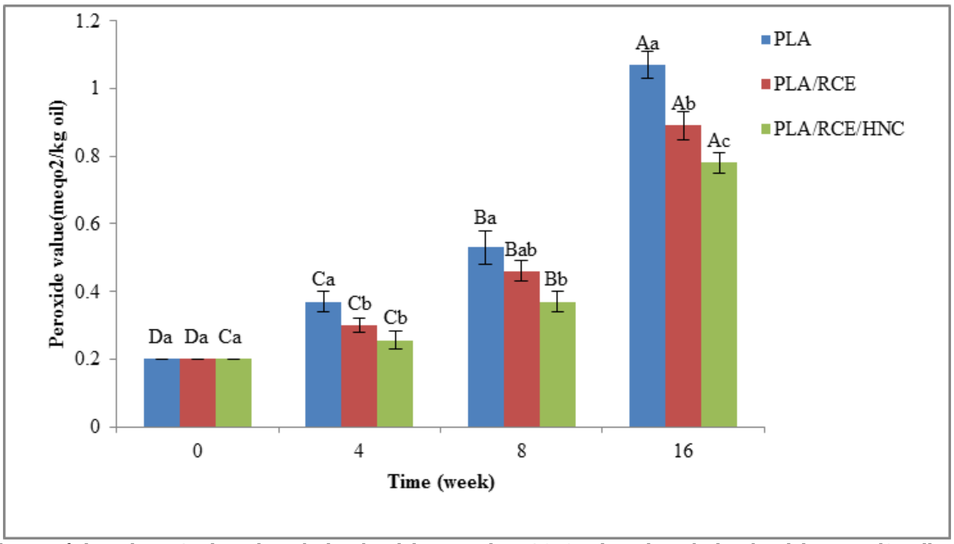

Fig.1. The PV of almonds. PLA: almonds packed with polylactic acid. PLA/RCE almonds packed with polylactic acid/roselle calyx extract PLA/RCE/HNC almonds packed with polylactic acid/roselle calyx extract/halloysite nanoclay clay. Different small and capital letters represent significant difference among means±SD of various films and one specimen during conservation respectively.

Thiobarbituric acid value

Fig. 2. displays the effect of RCE and RCE/HNC during 16 weeks of conservation on the TBA value of almonds. The use of RCE and HNC had a significant impact on the TBA value of almonds ($p<0.05$). The

findings indicate that the TBA of almonds had the enhancing trend during conservation; there was significant between PLA, PLA/RCE, and PLA/RCE/HNC on last week of preservation. The TBA of almonds packed with PLA was increased from 0.011 to 0.056 mg MDA kg^{-1} after 16 weeks of conservation.

It can be found (Fig. 2.) that in all kernels, the TBA change of almonds packaged with PLA/RCE/HNC is lower than other almond samples.

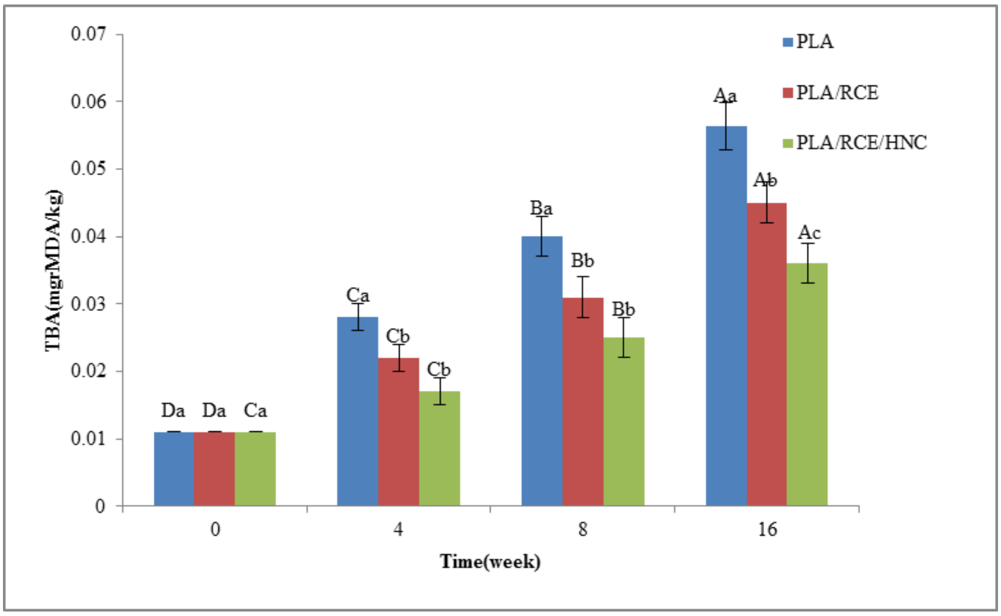

Fig.2. The TBA value of almonds. PLA: almonds packed with polylactic acid PLA/RCE almonds packed with polylactic acid/roselle calyx extract PLA/RCE/HNC almonds packed with polylactic acid/roselle calyx extract/ halloysite nanoclay clay. Different small and capital letters represent significant difference among means±SD of various films and one specimen during conservation respectively

Total mold and yeast count assay

Change in the total mold and yeast count is represented in Fig 3. The initial total mold and yeast count of almonds packaged with PLA film was 3.05 CFU g^{-1}. It reached 4.12 CFU g^{-1} for almonds packaged with PLA after 16 weeks of storage. The total mold and yeast count of all almonds increased during preservation, and the increase in almonds packaged with PLA film was highest. The mold and yeast count assay results displayed significant difference ($p < 0.05$) between the PLA film and PLA/RCE, PLA/RCE/HNC samples during conservation. At the last of the preservation, the lowest mold and yeast count of almonds were found in samples packaged with RCE/HNC. Total mold and yeast count achieved maximum levels of 3.77 in almonds packaged with PLA/RCE/HNC. Biofilm based on PLA PLA/RCE/HNC represented the highest antifungal activity.

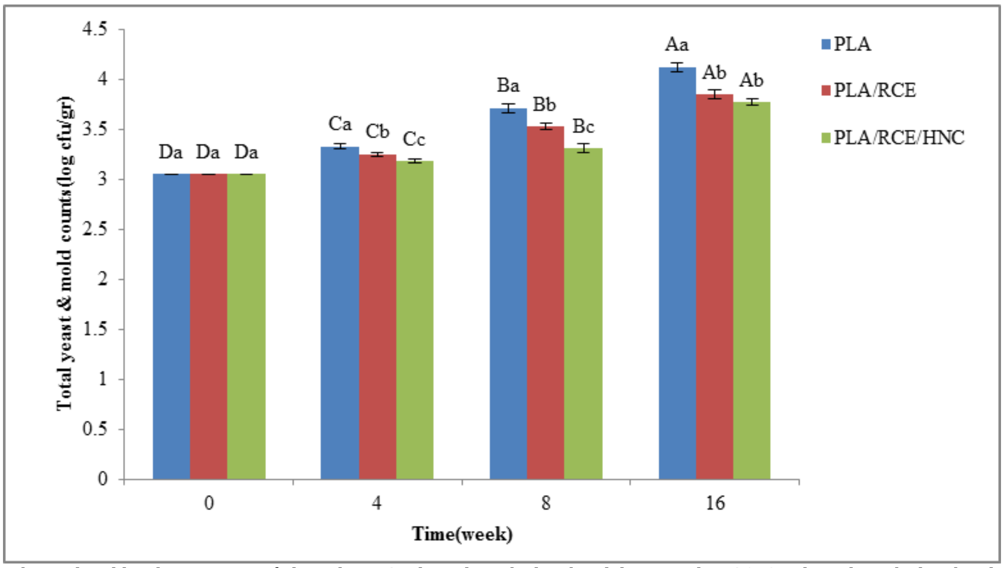

Fig.3. The total mold and yeast count of almonds. PLA: almonds packed with polylactic acid PLA/RCE almonds packed with polylactic acid/roselle calyx extract PLA/RCE/HNC almonds packed with polylactic acid/roselle calyx extract/ halloysite nanoclay clay. Different small and capital letters represent significant difference among means ± SD of various films and one specimen during conservation respectively.

Sensory properties

Sensory attributes changes in almonds were studied for 16 weeks, and the data are displayed in Fig. 4. The sensory attributes of all almonds decreased during preservation, and the decrease in pure samples was highest. After 16 weeks of preservation, color, and flavor scores were found as 3 and 2.5 respectively in the neat sample, whereas the color and flavor of the almonds packaged with RCE/HNC were measured as 4.5 and 3.5 respectively. The minimum color and flavor score was related to the control samples, and they were significantly ($P < 0.05$) different from the sensory attributes of treatment PLAs.

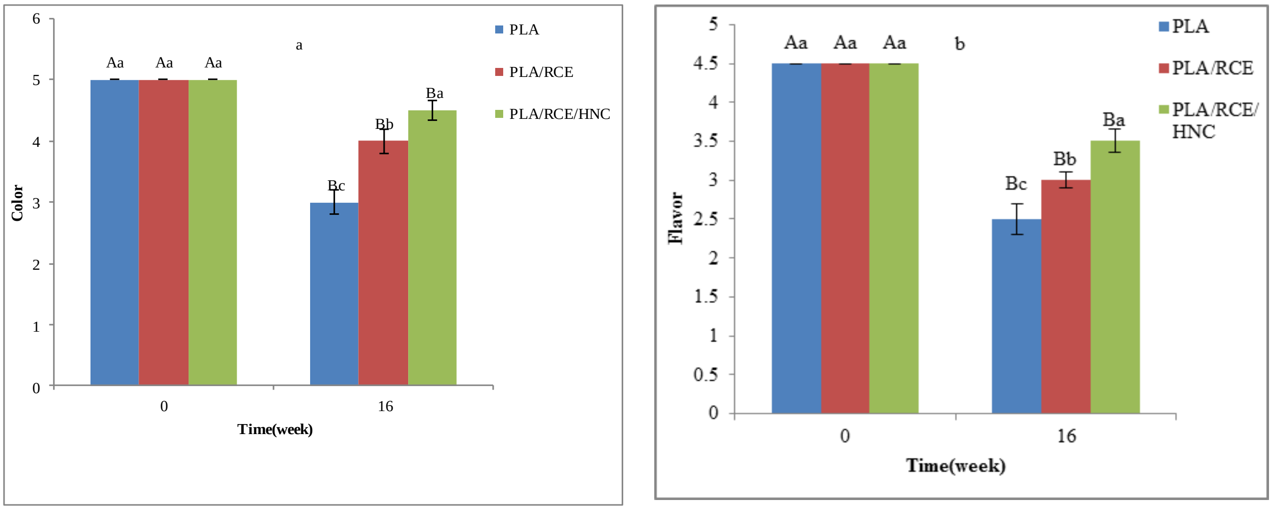

Fig.4. Color(a) and flavor (b)of almonds PLA: almonds packed with polylactic acid PLA/RCE almonds packed with polylactic acid/roselle calyx extract PLA/RCE/HNC almonds packed with polylactic acid/roselle calyx extract/halloysite nanoclay clay. Different small and capital letters represent significant difference among means±SD of various films and one specimen during conservation respectively.

Discussion

Hydroperoxides are the products of oxidation reactions, and the PV value is used to evaluate the concentration of these oxidation products. (Marvizadeh *et al*., 2014, Marvizadeh *et al*., 2017).

The impact of RCE and HNC was studied on the PV of almonds for 16 weeks (Fig. 1). Obtained findings show that the initial PV was 0.2 meqo_2 kg^{-1} oil while Farooq *et al*. (2021) demonstrated 0.24 meqo_2 kg^{-1} oil for samples. Whereas, Guiné *et al* Almeida and Correia (2014) represented higher PV for fried almonds, namely 9.4 meqo_2 kg^{-1} oil before conservation. According to Fig. 1. The almonds packaged with extract/nanofiller displayed the lower PV which is consistent with the investigation of Andrade *et al.* (2023). They stated that PLA film decreases the PV of almonds compared to pure samples.

This finding exhibits the desirable-preventing impact of HNC against O_2 traveling between almonds and the surrounding atmosphere.

RCE and HNC act as a good barrier against oxygen migration by enhancing the barrier attributes of the films based on PLA against O_2 (Marvizadeh *et al*., 2016, Sadeghi *et al*., 2023). The barrier properties enhanced after the incorporation of HNC related to the addition of nanofiller in the tortuous pathway of PLA film for oxygen to pass through (Fallah *et al*., 2022, Marvizadeh *et al*., 2018). Also, the barrier characteristics improvement in biofilm with RCE might be related to enhance in the interactions between PLA and extract (Sadeghi *et al*., 2023).

Fig 2. displays the TBA value, during preservation. The TBA value of almonds was enhanced significantly during 16 weeks maximum. The highest TBA value of almonds (0.056 mg MDA kg^{-1}) was attributed to the almonds packaged with pure film during preservation. Data of TBA value accord with data from Farooq *et al*. (2021), in which the initial value of TBA was 0.014 mg MDA kg^{-1} for samples.

Padehban *et al*. (2018) demonstrated an enhancement from 0.010 to 0. 056 meq O_2/kg at the end of conservation. Also, Faruk Gamlı and Hayoğlu (2007) describe TBA value's pistachio nut paste of 0.04 mg MDA kg^{-1} after 5 months of conservation. The lowest TBA value of almonds was attributed to the PLA/RCE/HNC. Nanofillers have displayed behavior as an excellent barrier to O_2 traveling in nuts packaged with cassava starch/bovine gelatin/titanium oxide nanoparticle/fennel essential oil (Chavoshi *et al*., 2023). Almonds packaged with polyethylene/nano-silver reduced the oxidation reactions of almonds packed with 3% nanoadditive compared to pure polyethylene (Tavakoli *et al*., 2017). The findings of TBA value were in accord with the investigation of Kazemi *et al*. (2020) that describe the oxidation stablity of pistachio was increased significantly upon the incorporation of nano-ZnO.

The initial total mold and yeast count of almonds was 3.05 log CFU g^{-1} (fig 3), which was enhanced in all samples during preservation. The greatest enhancement was found in almonds packaged with PLA while the least changes were almonds packed with RCE/HNC.

There are various indices, that can influence on fungi growth, such as RH, temperature, moisture content, and level of O_2 pressure. The biofilm decreases the population of mold by reducing O_2 transfer (Chavoshi *et al*., 2023, Fallah *et al*., 2024). The active film with nanoparticles/RCE has strong barrier against oxygen molecules and reducing the mold and yeast count by reducing O_2 traveling (Sadeghi *et al*., 2023). Also, the antifungal behavior of compounds in the roselle extract constitute a potential change for monitoring the fungal population in food production. Antifungal behavior has been related to polyphenolic compounds (Quansah *et al*., 2021). The mold count of walnuts was measured by Tavakoli *et al*. (2017). They demonstrated that the initial mentioned index is 2 log CFU g^{-1} on first day in polyethylene/nano-silver packaging. Walnuts were packed with nanoparticles exhibits higher antifungal behavior compared with the neat polyethylene. In another investigation on walnuts packed with tapioca starch/bovine gelatin, it was stated that mold and yeast count was highest in comparison with walnuts packed with tapioca starch/bovine gelatin/RCE/nano-ZnO during conservation (Aghayan *et al*., 2024). These results were in line with El-Sayed *et al*. (2020) who observed that active films based on RE/nano filler-ZnO and RE exhibit good antibacterial properties which delay contamination bacteria in packaged Ras cheese as compared to pure Ras cheese.

Sensory attributes were carried out by 9 members of panelists. They evaluated two indices from 1 to 5: color, and flavor after 16 weeks of storage. The sensory attributes of almonds is the key factor in nuts biopackaged and is influenced by the oxidation stability and constituent compounds. The data displayed that, the use of RCE/HNC by barrier characteristics against oxygen travel into the almonds increases oxidation stability and improves sensory properties. This is similar to the results in walnut kernels (Pakrah *et al*., 2021).

The almonds packaged with PLA reached 3 on week 16. However, the color score of PLA/RCE/HNC was 4.5 on week 16 (Fig. 4a). Color score in almonds decreased with enhanced preservation period, as Vera *et al*. (2018). Also, they exhibited that the color score (after 9 weeks) of almonds is 2 in pure specimens. In another investigation, sensory attributes displayed significant differences between control samples and pistachio nuts packed with nano particle-ZnO/cinnamon essential oil (Kazemi *et al*., 2020).

Conclusions

The effect of films based on PLA, PLA/RCE, and PLA/RCE/HNC was studied on mold contamination, sensory quality, and TBA and PV value of almonds. The PV and TBA values of specimens decreased significantly ($P < 0.05$) on last week of preservation, as a result of PLA film with RCE/HNC. The total mold and yeast count of the almond kernels packaged with PLA/RCE/HNC was significantly ($P<0.05$) lower than neat sample. Also, PLA/RCE/HNC film created an excellent effect on the flavor and color of almonds, and panelist's acceptances for almonds packaged with PLA containing RCE/HNC were higher than in the pure sample. According to the results, almonds packaged with PLA/RCE/HNC can provide a desirable protective style for preserving almonds.

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Conflict of interests

The authors declare that there is no conflict of interest.

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